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Habitat requirements and ecological niche of two cryptic amphipod species at landscape and local scales

Michael Eisenring,[1,5,†] Florian Altermatt,[1,2] Anja Marie Westram,[3] and Jukka Jokela[1,4]

[1]*Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology, Eawag, Überlandstrasse 133, CH-8600 Dubendorf, Switzerland*
[2]*Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland*
[3]*University of Sheffield, Animal and Plant Sciences, Western Bank, Sheffield S102TN UK*
[4]*Department of Environmental Systems Science, Institute of Integrative Biology, ETH Zurich, Universitätstrasse 16, 8092 Zurich, Switzerland*

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Abstract. Cryptic species are phylogenetically diverged taxa that are morphologically indistinguishable and may differ in their ecological and behavioral requirements. This may have important implications for ecosystem services and conservation of biodiversity. We investigated whether two ecologically important cryptic species of the freshwater amphipod *Gammarus fossarum* (types A and B) are associated with different habitats. We collected data on their occurrence at both the landscape scale (large watersheds) and at the local scale (river reach) to compare macro- and microscale environmental parameters associated with their presence. Analysis of the landscape scale data showed that occurrence of types A and B differ with respect to watershed and river size and, interestingly, human impact on river ecomorphology. Whereas type B was mainly found in less forested areas with higher human impact, type A showed the opposite occurrence pattern. Analyses of the local scale data suggested that habitats occupied by type A were characterized by larger gravel, larger stones and less macrophytes than habitats occupied by type B. The landscape and local data set showed contradicting patterns with regard to stream size. Overall, the observed differences between the two types of *G. fossarum* most likely reflect ecological differences between them, but alternative explanations (e.g., historical colonization processes) cannot be completely ruled out. Our study underlines that common cryptic species can differ in their ecology and response to anthropogenic influence. Such differences in habitat requirements among difficult-to-identify taxa present a challenge for biodiversity and ecosystem management. Our results emphasize the importance of conservative and precautionary approaches in maintenance of habitat diversity and environmental heterogeneity.

Key words: Cryptic species; ecological niche; environmental correlates; *Gammarus fossarum*.

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[5] Present address: Agroscope Institute for Sustainability Sciences, Reckenholzstrasse 191, 8046 Zurich, Switzerland.
† **E-mail:** michael.eisenring@agroscope.admin.ch

Introduction

With the advent of powerful molecular genetic methods it is possible to identify phylogenetically distinct, but morphologically similar species. The rate of discovery of cryptic species has increased exponentially over the past decades, revealing the magnitude of this previously undiscovered diversity (e.g., Bickford et al. 2007 and references therein). As morphological

similarity does not necessarily imply ecological similarity, assessment of ecological niche differences among cryptic species is crucial for many research fields such as biological control and use of biological indicator taxa (Feckler et al. 2012, 2014). Cryptic species may differ with respect to their ecological niches (e.g., Bidochka et al. 2001, Davidson-Watts et al. 2006) and may interact differently with their environment (Bickford et al. 2007). Newly discovered cryptic species may therefore have important implications for conservation of biodiversity where the goal often is to define and protect endemic species richness (Bickford et al. 2007).

The arthropod order of Amphipoda lends itself to the study of ecological differences between cryptic species, as its members play several important roles in aquatic ecosystems (MacNeil et al. 1997, 1999, Duffy and Hay 2000) and often contain complexes of cryptic species (e.g., Wellborn and Cothran 2004, Witt et al. 2006, Lefébure et al. 2007, Fišer et al. 2015). Of specific significance is the freshwater keystone amphipod *Gammarus fossarum*, which consists of several cryptic species, some of them probably millions of years old (Scheepmaker and Van Dalfsen 1989, Müller 2000, Weiss et al. 2014). Reproductive isolation of the cryptic *G. fossarum* species is indicated by the absence of intermediate genotypes in mixed populations (Müller 2000). *G. fossarum* has a central position in food chains of prealpine streams, processing dead organic material and representing a major source of fish nutrition (MacNeil et al. 1997, Dangles et al. 2004). Furthermore, *G. fossarum* is used as an indicator organism for habitat quality and ecotoxicological studies (e.g., Lukancic et al. 2010, Bundschuh et al. 2011, 2013). In headwaters of our study area (Switzerland) *G. fossarum* is frequently the dominant benthic macroinvertebrate (Altermatt et al. 2014), and loss of these populations may have pronounced effects on the ecosystem (MacNeil et al. 1997, 1999).

Despite the ecological importance of this species complex, relatively little information regarding ecological requirements of *G. fossarum* is available (but see Meijering 1991, Peeters 1998, Stürzbecher et al. 1998, Müller et al. 2000). This is especially true for potential differences between the cryptic *G. fossarum* species. Given their age and the potential for evolution in distinct refugia during Pleistocene glaciations (Müller 2000), differences in various biological traits due to divergent selection (and drift) are conceivable. Indeed, a few studies have found differences in biological characteristics including sensitivity to toxic chemicals (Feckler et al. 2012), infection with parasites (Westram et al. 2011*a*), and timing of reproduction (Stürzbecher et al. 1998).

The most common cryptic species within the *G. fossarum* complex, namely types A and B according to the terminology of Müller (1998), have geographically distinct distribution patterns in Europe. In Switzerland, their distribution ranges overlap and sympatric populations occur. Whereas type A is common in the eastern parts of Switzerland, type B is more frequent in the western parts (Altermatt et al. 2014). Several streams in the Rhine drainage are known where both species occur in sympatry (Westram et al. 2011*b*, Altermatt et al. 2014).

Preliminary evidence suggests that types A and B might have different preference of microhabitats (e.g., Stürzbecher et al. 1998, Müller et al. 2000). Müller et al. (2000) classified streams in Germany into two categories: streams with plant-rich substrates and mud versus streams characterized by gravel and the presence of leaf litter. Type B mostly occurred in lower altitude streams of the former category, whereas type A did not show a clear tendency. These differences in ecological niche were detected albeit the study restricted to relatively crude habitat categorizations, and very little information was available on microhabitat associations within streams. However, a study on a single mixed population indicated that type A was mostly associated with stones, whereas type B was more common in areas with macrophytes (Stürzbecher et al. 1998). Our motivation was to expand these studies to more detail on microhabitat occupation across a large number of streams and ask if the reported differences in ecological niche are general enough to conclude that the species are ecologically different.

We analyzed environmental factors to characterize habitat occurrence pattern of the two cryptic species, *G. fossarum* types A and B, in Switzerland both at the landscape scale (watershed) and at the local scale (river reach). The landscape data include a large number of stream characteristics and parameters describing anthropogenic effects. On the local scale, we quantified various

microhabitat types within each stream and associated them with *G. fossarum* occurrence. Our study allows for a general test of environmental correlates of distribution of *G. fossarum* species, and evaluation of potential ecological niche differences between the two cryptic species. A detailed description of the distribution patterns of cryptic *G. fossarum* species is of high relevance for adjusting its future role as a biological indicator species and will add to our understanding of fundamental ecological processes in prealpine headwater streams.

Methods

Field survey

Landscape scale.—We used data on the occurrence (presence/absence) of *G. fossarum* within the River Rhine drainage in Switzerland. The data are based on standardized and representative sampling program conducted within the project "Biodiversity Monitoring in Switzerland" (BDM, 2009, 2014, Altermatt et al. 2013). In this project, all macroinvertebrates are monitored on a systematic sampling grid covering the whole of Switzerland. At each site, specifically trained field biologists collected and identified macroinvertebrates to species level, using well-established and highly standardized methods. The sampling sites are randomly chosen across Switzerland, and the data sampled therein thus reflect a representative depiction of both biological (e.g., occurrence of species) and abiotic (e.g., altitudinal distribution) variables at the landscape scale (see also Stucki 2010, Altermatt et al. 2013). Observed differences can thus be directly linked to biological differences in the two species or colonization events. We analyzed all 222 sampling localities within the Rhine drainage (36,500 km^2) that were sampled once between 2010 and 2012 (Fig. 1a). The BDM sampling scheme includes watercourses higher than 1st order streams (Stucki 2010). Standing waterbodies, first-order streams, and watercourses completely inaccessible by wading are not included in the BDM program for methodological reasons (Stucki 2010, BDM 2014). The sampling scheme specifically considers the representative inclusion of headwaters, which are naturally much more numerous (Altermatt 2013). Sampling sites are located between 280 and 2718 m a.s.l. All sites together reflect the Swiss watercourses and amphipod species diversity therein. The seasonal timing of the sampling was optimized for the local phenology of macroinvertebrates (Stucki 2010) and took place between March and July. For each site, we collected information on the presence of *G. fossarum* A and B (Altermatt et al. 2014)

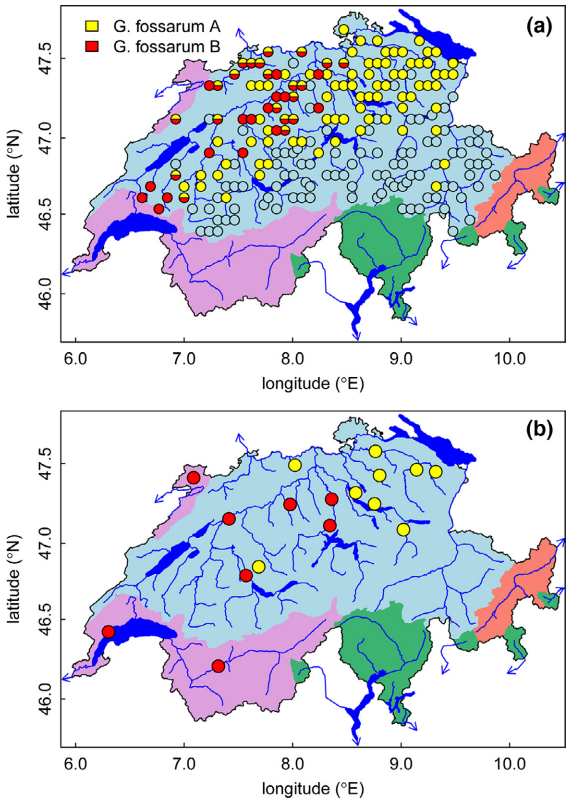

Fig. 1. (a) Landscape scale: overview of the occurrence of *Gammarus fossarum* A and B within the River Rhine drainage (light blue shading) in Switzerland. All 222 BDM sampling sites are depicted on the map, and reflect the occurrence distribution of river sites across the whole drainage basin. The (co-) occurrences of the two types of *G. fossarum* types A and B are depicted in yellow and red, respectively. Open circles reflect sampling sites without *G. fossarum*. (b) Distribution of all sampled *G. fossarum* populations in Switzerland contributing to the local scale data set. Main rivers and lakes are given as dark blue lines/polygons for orientation and drainage basins are color-coded: Rhine drainage (light blue), Rhone drainage (violet), Ticino/Inn drainage (green).

and on environmental variables (Stucki 2010). The environmental variables that were evaluated for each sampling site are presented in Table 1, for details of the sampling methods, see Stucki (2010).

We identified amphipods based on morphology using standard identification literature (Eggers and Martens 2001). We further used previously established microsatellite markers for separating *G. fossarum* A from *G. fossarum* B. We extracted DNA from 5 to 50 individuals of the *G. fossarum* complex per site (either extracting DNA from whole individuals or from pereopods) and analyzed 10 microsatellite markers using the method described in Westram et al. (2010). Specific allelic combinations in these 10 microsatellite markers have been described as a diagnostic tool to tell types A and B apart (for details see Westram et al. 2010, 2011*b*, 2013). The microsatellite markers diagnostic for type A is gf27 polymorphic with alleles >200 bp (but ≠205), whereas for type B the marker is monomorphic at 205 bp. The BDM method is not optimized for a quantitative sampling of macroinvertebrates, and we thus only used presence/absence data.

Local scale.—Gammarus fossarum specimens were collected from 17 different streams in Switzerland during August and October 2010. (Fig. 1b). The streams were inhabited either only by types A or B, as known from previous studies based on molecular markers (Westram et al. 2013, Altermatt et al. 2014, A. M. Westram et al., *unpublished data*). To distinguish microhabitat characteristics of A and B streams and to investigate potential microhabitat preferences of the two *G. fossarum* species, each stream was examined on a total length of 30 m, subdivided into 15 sections of 2 m length each. Exceptions were three stream sites where only 10, 11, and 14 sections were defined due to spatial restrictions. For each section the proportions of six microhabitat types were estimated by eye and channel width and flow velocity were measured (Table 2). A quantitative sample of benthic invertebrates was taken from a random position within each section using a HESS-sampler (sample surface area = 452.4 cm^2, mesh size 0.5 mm). As an exception from random sampling, rare microhabitats were sampled at least once per location, without paying attention to randomization. Gammarids of a sample were washed out in a sieve (mesh size 1.5 mm) and presence/absence was recorded. Because of the mesh size, only individuals with a body size of 5 mm or more were included in the study.

Table 1. Environmental variables that were measured at the landscape scale. Dimensionality of the continuous-scale variables was reduced with principal component analysis (PCA) to three variables (PCA1–PCA3). Remaining categorical variables were included in the multinomial logistic regression model directly together with the three PCA variables. PCA columns show component loadings for each continuous variable on each PCA axis and the proportion of variance explained by each PCA axis (on the top row with label).

Description	Measurement unit/notes	Analysis	PCA1 (31%)	PCA2 (25%)	PCA3 (14%)
Catchment size	km^2, ln transformed	PCA	0.813	0.098	0.309
Channel depth	m, ln transformed	PCA	0.766	0.300	0.090
Altitude	m	PCA	−0.218	0.894	−0.179
Proportion calcite in the watershed	Proportion of calcite cover (vs. noncalcite) in the whole catchment area	PCA	0.020	0.268	−0.536
Proportion agriculture in the watershed	Proportion of agricultural area in the watershed within a 1 km buffer	PCA	0.309	−0.769	−0.307
Proportion forest in the watershed	Proportion of forested area in the watershed within a 1 km buffer	PCA	−0.489	−0.099	0.742
Channel width	m, ln transformed	PCA	0.780	0.420	0.162
Coverage of algae at the site	No, medium, high	Factor			
Human impact on ecomorphology	No impact, low impact, high impact	Factor			
Soft sediments (no, medium, high)	High group too rare, pooled to medium	Factor			

Table 2. Characterization of microhabitat parameters that were used to classify stream sections at the local scale.

Type	Description
Stones	Objects with a size of 3–15 cm
Gravel	Objects with a size of 0.5–2 cm
Large gravel	Mix between Gravel and Stones
Large stones	Continuously stony surface bigger than the sample area of the HESS-Sampler (452.4 cm^2)
Dead organic material	Dead plant material, especially leaves
Mosses and macrophytes	Living plant material such as roots, aquatic plants, grass or mosses
Flow	Velocity of stream (m/s): low = 0–0.3, medium = 0.31–0.6, high = 0.61+
Channel width	Width of channel section (m)

Data analysis

Landscape scale.—We grouped the 222 biodiversity monitoring sites into four classes according to presence–absence of *G. fossarum* types A or B (no *G. fossarum*, types A and B, or both). Next we tested how these categories associated to environmental and biological parameters that were measured at the sites. We first reduced the dimensionality in the continuous-scale environmental variables using a principal component analysis (PCA) (Table 1). We then used multinomial logistic regression where the dependent variable was the *G. fossarum* classification and independent covariates (continuous-scale) were the two statistically significant PCA axes. As additional categorical independent factors we used the ecomorphology-based evaluation of the human impact at the sites, soft–sediment abundance class, and classification of algal coverage (Table 1). We evaluated the model using a forward–stepwise evaluation of independent variables excluding interactions between factors.

Local scale.—Our local scale data set consists of environmental parameters from a total of 245 sections from 17 study streams. We also included sections that had no *G. fossarum* to get a reference of the environment that was neither preferred by *G. fossarum* A nor B.

We first reduced the dimensionality in the environmental parameters with a categorical PCA (Linting et al. 2007) to three dimensions, using each section as an independent sample. We then used the object scores of the three dimensions to ask if type A of B were associated with any specific habitat dimensions. To test for differences in habitat characteristics between the two *G. fossarum* types we used a multivariate response profile analysis (MANOVA) where object score profiles of types A and B presence were compared to each other and to samples with no *G. fossarum*. We did not include stream identity in the statistical analyses because in each stream we found only one type of *G. fossarum*, therefore stream identity as a factor would have been confounded with *G. fossarum* type. For completeness we ran the same statistical models using stream as a factor instead of *G. fossarum* type and present the results graphically for comparison.

All statistical analyses were conducted with SPSS version 22.

Results

Environmental correlates of Gammarus fossarum *types A and B distribution in the landscape*

We had data on the *G. fossarum* presence–absence and all environmental variables from 222 BDM sites. At 75 of these sites we found *G. fossarum* A, at 17 sites we found *G. fossarum* B. The two types of *G. fossarum* were co-occurring at 27 sites. No *G. fossarum* was found at 102 sites.

The three first PCA axes captured 70% of variation in the data. Factor loadings (Table 1) show that the first axis (31%) loaded positively to channel and catchment size and negatively to proportion of forest in the area. The second axis (24%) loaded positively to altitude and negatively to proportion of agricultural land in the area, whereas the third axis (15%) loaded positively on proportion of forest in the area. Overall, *G. fossarum* A was found between 280 and 1620 m a.s.l., whereas *G. fossarum* B was found between 280 and 867 m a.s.l.

The multinomial logistic regression model gave us several interesting insights on how *G. fossarum* presence–absence relates to environmental parameters and how the predicted occurrence patterns differed between the *G. fossarum* types. Two of the three PCA axes had a significant association with the presence of *G. fossarum* in the final multinomial logistic regression model. PCA2, which captured largely variation in altitude, entered the model first, being the most significant

($P < 0.001$) explanatory variable, whereas PCA1, which captured variation in size of the watershed and river size, entered second ($P = 0.010$). Interestingly, human impact on ecomorphology (MSK) remained as the only significant factorial variable in the final model. Effect of human impact on morphology was weaker than that of the two principal components, but still statistically significant ($P = 0.035$).

More detailed interpretation of the model brings three main results. First, high elevation sites (which are also characterized by less agricultural use) are less likely to support *G. fossarum* populations (Fig. 2). This is shown by very low predicted occurrence probabilities for sites where PCA2 scores are high. Predicted probabilities for both *G. fossarum* types declined at a similar rate as a function of PCA2, indicating that species responded similarly to variation captured by PCA2.

The second main result concerns differences among *G. fossarum* types in response to PCA1, which mainly corresponds to size of the watershed and size of the river. Occurrence probability of type B increases with PCA1 score, suggesting higher likelihood to find type B populations in larger and less forested rivers (Fig. 2b). On the contrary, type A has much higher occurrence probabilities for low values of PCA1 (forested, smaller streams) (Fig. 2a).

The third interesting result is that species response to human impact seems to be opposing for the two types. Type A occurrence probabilities are highest for near natural sites, whereas they were lowest for type B (Fig. 2).

Environmental correlates at the local scale

The first three dimensions of the categorical PCA analysis captured 61% of total variance among the environmental parameters. Highest object scores for the first dimension (27% of variance) were associated with samples that had high proportion of large gravel substrate and wide channels, characterizing larger streams, with less large stones and dead organic material (Fig. 3). The second dimension (19% of variance) was associated positively to macrophytes and slow flow, separating samples with macrophyte habitat. The third dimension (15% of variance) associated positively on large stones and gravel, characterizing sections with heterogenous habitat. Distribution of samples along these habitat dimensions is shown in Fig. 4a,c and e.

Profile analysis suggests differences in all three habitat dimensions between presence of the two *G. fossarum* types (Figs. 4 and 5) (Wilks' Lambda = 0.56, $F_{4,482} = 40.1$, $P < 0.001$). Stream sections where type A was found were characterized by wider channels, more large gravel and large stones and less macrophytes than sections with type B (Fig. 4). Sites where no *G. fossarum* were found were fastest flowing sections with largest stones and gravel (Fig. 4).

Discussion

We investigated environmental parameters associated with presence of *G. fossarum* populations. In the following, we discuss potential ecological and historical factors driving *G. fossarum* distribution and especially focus on differences between the two cryptic *G. fossarum* species A and B in this respect. Our data contribute to the growing body of research showing that cryptic species, although morphologically indistinguishable, can differ in ecological characteristics (Narins 1983, Henry 1994, Feulner et al. 2006, Westram et al. 2011*a*, Cothran et al. 2015). Since *G. fossarum* is one of the most common macroinvertebrate species in prealpine headwater streams of central Europe, the understanding of the ecological differences between these cryptic species are of high importance and have implications for the conservation of freshwater ecosystems.

Environmental parameters associated with occurrence of the Gammarus fossarum *complex*

We found several key parameters that are associated with the presence of *G. fossarum*. Some of them affect both species similarly, but others indicate differences between the two species.

Our analyses show that altitude is a key factor determining *G. fossarum* distribution (Fig. 2). Sites above about 1600 m a.s.l. seem not habitable for both *G. fossarum* types. This can probably be attributed to harsher climatic conditions and lower food availability (i.e., fewer decaying leaves) in high-altitude compared to lowland

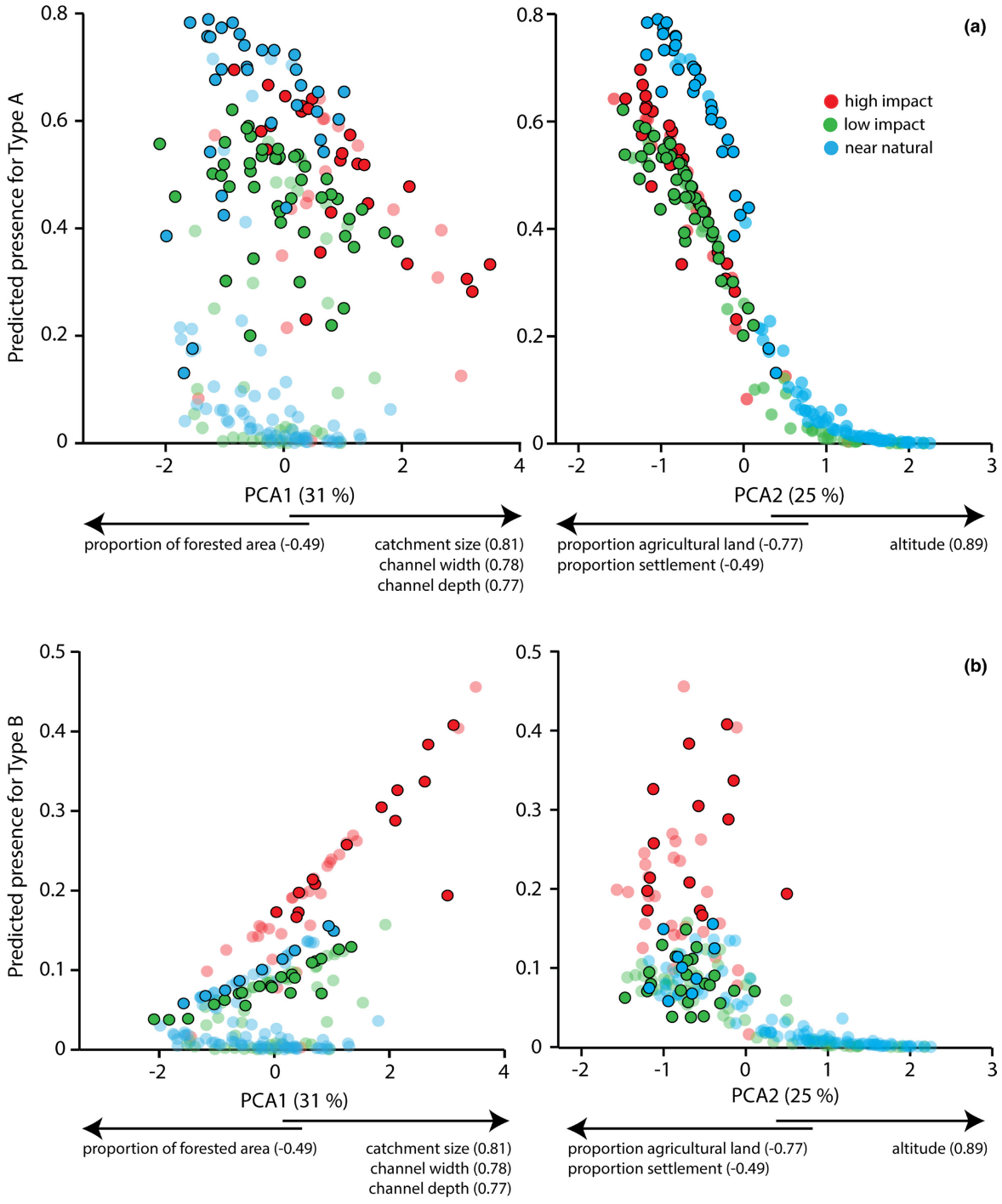

Fig. 2. Predicted probability for presence of *Gammarus* types A (a) and B (b) based on a multinomial logistic regression using PCA1 and PCA 2 as independent variables. Points with black edge and darker color show sites where either type of *G. fossarum* was found. Arrows below the *x*-axis show the direction of loading of environmental variable in each of the two PCA axes.

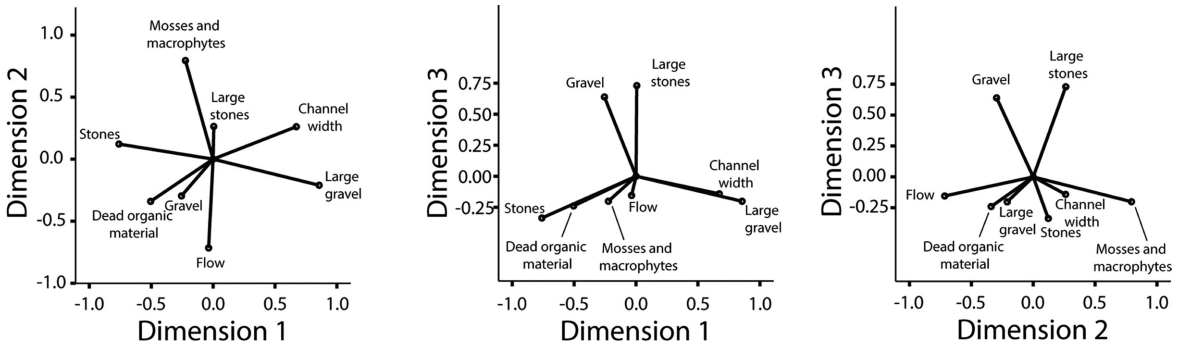

Fig. 3. Loading of environmental variables on the three Categorical PCA axes.

streams. Furthermore, *G. fossarum* generally avoided fastest flowing stream sections with large stones and gravel. Both species occurred with high probabilities in stream sections in agricultural land or settlement. This can potentially be explained with the comparatively high nutrient levels of these stream sections and therefore a high amount of submerged aquatic vegetation, which mainly positively correlates with aquatic invertebrate abundance (Krull 1970, Anteau et al. 2011). Interestingly, however, human impact, characterized *via* the MSK classes, had clearly different effects on the distribution patterns of the two cryptic *G. fossarum* species. Whereas type A mostly occurred at near natural sites, the opposite pattern was found for type B. As the MSK classification can correlate with water pollution (or human influence in general), these differences may be linked to differences in physiochemical tolerances of the two species. Indeed it has been demonstrated that type A shows a higher overall sensitivity toward specific insecticides and fungicides compared to type B (Feckler et al. 2012). However, since type A was not negatively affected by the proportion of settlement and agricultural land, differences in physiochemical tolerances can probably be ruled out as an explaining factor. Based on MSK classifications, stream sections that are under high human impact are often characterized by a high degree of artificial ground modifications and compaction (Stucki 2010). This may affect the abundance of aquatic vegetation of certain fish and macroinvertebrate predators as well as intra- and interguild cannibalism and competition with multiple direct and indirect effects for *G. fossarum* population dynamics (MacNeil et al. 1999, Anteau et al. 2011 and therein). Type A might react differently to these changes in vegetation, predator and competitor abundance than type B, and therefore show different patterns regarding MSK characterization. This hypothesis is partially contradicted by the findings of Seymour et al. (2016) who found no covariation between macroinvertebrate diversity and the genetic diversity within co-occurring *G. fossarum.*

Our landscape scale data show that type B occurs with a higher probability in large streams and less forested areas, whereas type A tends to the opposite pattern but is showing a more generalist behavior regarding these environmental factors (Fig. 2). Similar results were found on the local scale. Type A mainly occurred in streams that were comparatively rich in gravel and stones but poorer in macrophytes, whereas type B was mainly found in streams with typical grassland characteristics (high proportion of macrophytes but less dead organic material or stony substrate). Our results are in line with the study of Müller et al. (1998) in which type B was more often associated with grassland streams than woodland streams. Differences in the degree of feeding plasticity between the two types could explain some of the found distribution patterns. Friberg and Jacobsen (1994) state that species of the genus *Gammarus*, which are generally characterized as typical detrivore-shredders, are often able to exploit additional food sources such as fresh aquatic plant material. This could explain why type B was mainly found in streams with less dead plant material but with a higher proportion of macrophytes compared to streams inhabited by type A. Furthermore, parasitism and predation may have a strong impact on *G. fossa-*

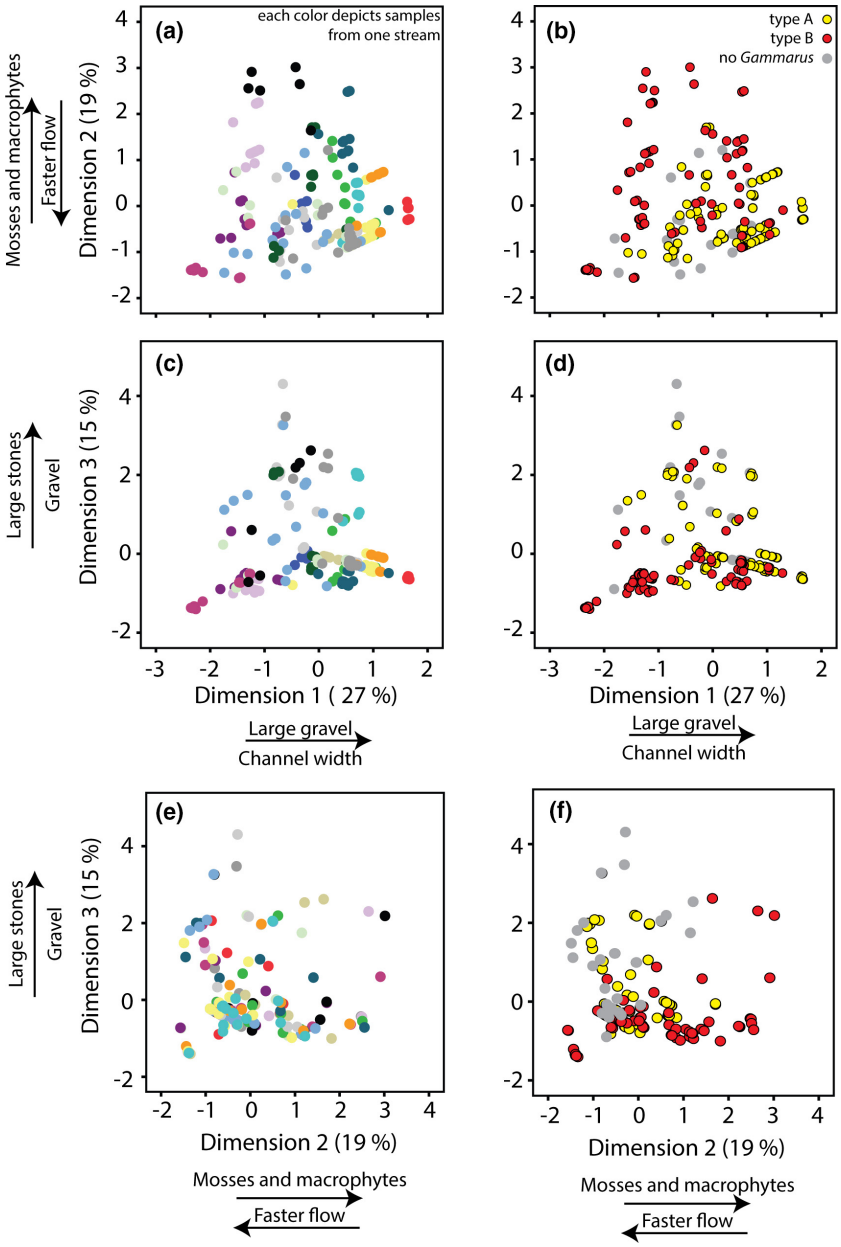

Fig. 4. Object scores of samples by stream (a, c, e) or by type of *Gammarus fossarum* (b, d, f) with respect to three Categorical PCA axes, with colors indicating either the sample stream (a, c, e) or *G. fossarum* type found (b, d, f).

rum population dynamics (MacNeil et al. 1999 and therein). Different predation or parasite pressure (Westram et al. 2011*a*), or differences in defensive behavior of the two types could therefore potentially lead to a selection for different environmental conditions in which the respective predation or parasite avoidance strategies are optimized. However, in order to get a better understanding of the microhabitat differentiations between *G. fossarum* types A and B, clearly more detailed local scale studies are needed, especially at sites were types A and B occur sympatrically.

Stream size was the only parameter that showed an opposite outcome on the local compared to the landscape scale. Based on our landscape scale model, type A was mainly

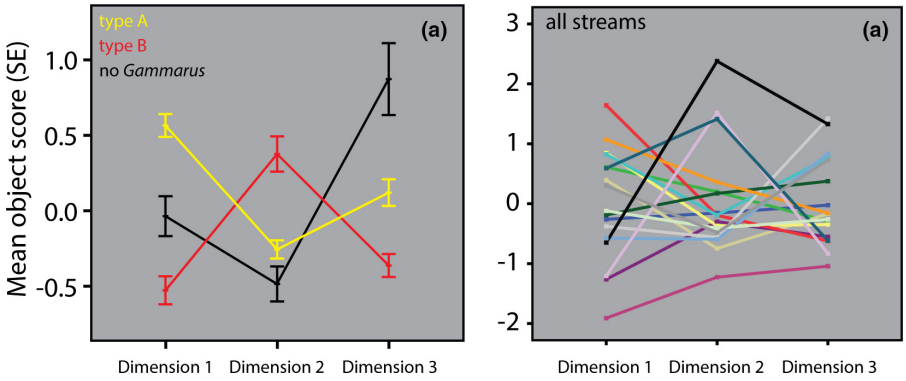

Fig. 5. Profile plots of mean object scores of the Categorical PCA dimensions for (a) each *Gammarus fossarum* type and (b) for each stream. See Fig. 3 for loading of environmental parameters on the CatPCA dimensions.

found in smaller streams than type B, whereas the local scale data suggest the opposite pattern. However, due to extreme differences in the range of river widths included in the landscape data (streams of all sizes were included) compared to the local data (only smaller streams were selected) comparisons of the two data sets regarding stream size have to be treated with caution.

The landscape and the local data set were not sampled at the same time. This raises the question regarding phenological effects biasing our results. Stürzbecher et al. (1998) conducted a study were they focused on seasonal differences in abundance and reproduction of *G. fossarum* A and B. The local data set was sampled during August and October, a time when according to Stürzbecher et al. 1998 the relative abundance between A:B is roughly 50:50. We did not have time-series data, and each site at the landscape scale data was sampled once between March and July. Sampling time was optimized with respect to the elevation (for details see Altermatt et al. 2013, Stucki 2010). While Stürzbecher et al. (1998) shows that numbers of A and B can fluctuate differently during that period of time, we do not think this is affecting our interpretations for two reasons. First, the sampling time was optimized for local phenology for all sites, such that similar phenological (but different Julian) data was compared. Second, our analysis is mostly focusing on presence/absence and not abundance data. Thus, even if there were changes in abundances, they were likely not strong enough to override the presence–absence patterns, and would also only be relevant for the relatively small number of sympatric populations.

Possible evolutionary and historical factors driving Gammarus fossarum *distribution*

The central finding of this study is that the two cryptic *G. fossarum* species are associated with different environmental parameters. In the following we discuss potential evolutionary and historical mechanisms that could alternatively explain the found distribution patterns.

Historical recolonization processes, rather than distinct adaptations, might partly explain the differences between the two species. A clear latitudinal distribution pattern of the two cryptic species was found for Switzerland (Fig. 1). Type A is more common in the eastern parts of the country; type B is more frequent in the western parts (e.g., Westram et al. 2011*b*, Altermatt et al. 2014). Other studies (e.g., Müller et al. 2000) imply that a similar distribution pattern is also true for Germany. Although both cryptic *G. fossarum* species diverged before or during the last glacial period in the Pleistocene and then probably came into contact due to the withdrawing of the glaciers (Scheepmaker 1990), a lack of time for a complete overlap of the two cryptic species could explain current distribution patterns. Alternatively, competitive exclusion between A and B species could have prevented the spread of the two species across the whole studied area and therefore be responsible for the separation we find nowadays. For example, intraguild predation is wide spread in freshwater gammarids (MacNeil et al. 1997,

1999 and therein) and can result in rapid species exclusion and replacement (Dick et al. 1993). In both cases, if the eastern and western parts of our study area differ with respect to environmental characteristics, the distinct species distributions could generate species-environment associations even if the two species are not differently adapted. We argue that this is unlikely to be the sole explanation. First, no obvious geological pattern that matches the distribution of the cryptic species of the *G. fossarum* complex in Switzerland can be found. The only environmental factor that changed with latitude and that was associated with *G. fossarum* distribution was altitude. Although it had a strong impact on *G. fossarum* distribution in general, we found that both *G. fossarum* types were similarly affected by altitudinal changes. Second, in the area where the distributions of the two species overlap, it has been found that species composition is clearly distinct even between geographically very close populations (few km) (Alp et al. 2012). A similar pattern was found for the area of overlap in this study (Fig. 1a). In these cases, local environmental differences seem more likely to explain distribution patterns than historical reasons.

We suggest that, rather than being explained by historical factors alone, the observed differences are partially explained by different adaptations of the two cryptic species. They existed in different refugia during the last glaciation for extended periods of time (Müller 2000). These refugia are in geographically very different regions, with potentially very different selection pressures. Therefore, it is quite plausible that distinct adaptations have evolved, with the two species specializing on different habitats. Future work should further investigate this, for example, using lab experiments to test for differential adaptation.

Implications for conservation management

The knowledge of cryptic species and the potential ecological and behavioral differences within such species complexes necessarily lead to new questions regarding conservation management strategies.

In this study, we found good evidence for ecological differences between the two cryptic *G. fossarum* species, A and B, which are often associated with different environmental parameters and differ in many habitat requirements. *Gammarus* species play a fundamental role in many freshwater ecosystems and drastic decreases of their populations can have severe consequences for other trophic levels. Besides the two studied species types A and B, several additional cryptic species, which also may differ in their ecology, exist within the *G. fossarum* complex (Müller 1998, Weiss et al. 2014).

In order to preserve *G. fossarum* populations it is therefore necessary not to preserve a single type of freshwater stream but to maintain a highly diverse set of heterogeneous stream types, as the distribution of cryptic *G. fossarum* species often can depend on different environmental factors.

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